

A CONTRIBUTION TO THE BIOLOGY OF THE
RINGWORM ORGANISM.

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It is not my intention in this paper to discuss the biology of the ringworm organism in its various phases, but simply to draw attention to some points concerning the production of ferments by the *Trichophyton*. The object of the research was to determine whether the ringworm organism in the course of its growth in various soils outside the body produces ferments, and, if so, what their nature is.

The nature of the ferments produced by the bacteria has been investigated by Bitter, Fermi, Lauder Brunton, the writer, and others. It has been found that those bacteria which liquefy gelatine do so by means of a soluble ferment. The action of this ferment on gelatine can be demonstrated apart from the cells that produce it. Thus, if one adds the liquefied gelatine alone to fresh gelatine, a liquefaction takes place. In a similar way it has been proved that the bacteria produce diastatic and milk-curdling ferments.

The present writer's investigations in this direction had hitherto been confined to the bacteria. It was with a view of extending these investigations to the group of the moulds, that the ordinary *Trichophyton tonsurans* was selected. Could one demonstrate the production of ferments by the moulds, as had already been done in the case of the bacteria?

The pure cultures of the trichophyton were obtained from Dr. Kral of Prague. They corresponded in their morphology and growth with the trichophyton associated with ringworm in the scalp in children. The morphology of the *Trichophyton* is not touched upon in this paper, a subject which is being so carefully worked out by Dr. Sabouraud and others.

Dr. Sabouraud believes that the *Trichophyton* organisms consist of different species, but that they all belong to the genus *Botrytis*. He describes two varieties of *Trichophyton* in ringworm, namely, the *T. microsporon* (in 60 per cent.), and the *T. megalosporon* (in 40 per cent.).

Rosenbach describes seven groups of *Trichophyton* isolated by him.

The full details will be found in the works these writers have recently published.¹

One thing at anyrate is certain, that the constitution of the soil on which they are grown exercises a considerable influence on their manner of growth. Their appearance, also, varies according to the temperature at which they are grown. In studying the morphology of the *Trichophyton* group, it is most important that the cultures be kept at a uniform temperature.

The first series of experiments were made to determine whether the *T. tonsurans* produces a peptonising enzyme.

Tubes of 10 per cent. gelatine were inoculated with the ringworm organism, and placed in an incubator at 20° C. The trichophyton in the course of its growth completely liquefied the gelatine. When liquefaction was complete, a few drops of the liquefied gelatine were transferred to the surface of sloping gelatine. The tubes were then placed at 20° C., a temperature at which the gelatine remained solid. The result was a distinct dimpling of the surface of the gelatine at those points where the liquid gelatine rested upon it. As the liquefaction advanced the gelatine became distinctly furrowed. The liquefied gelatine collected at the lower part of the tube. It remained quite clear, and showed no traces of growth. The gelatine from the ringworm culture had therefore liquefied fresh gelatine.

In a second series of experiments the sterile gelatine was first liquefied, and a few drops from a gelatine culture of ringworm were well mixed with it. The gelatine was then allowed to resolidify, and kept as before at 20° C.

The following day the tubes were removed from the incubator, and cooled down under the tap. It was found that in the short space of 15–17 hours, two-thirds of the gelatine had become liquid; only the lower third in the tube regelatinised on cooling. The liquefied gelatine remained clear and free from any visible growth. Here, again, there was evidence of a marked dissolving action on gelatine, independent of the growth of the organism itself.

A further series of experiments were made in the following manner:—

The liquefied gelatine from a ringworm culture was poured on the surface of sterilised gelatine in a test tube. A mark was made on the tube to indicate the line of demarcation between the two layers of gelatine. The tubes so prepared were again kept at 20° C. On examination, after 18 hours, it was found that 5 mm. of the sterile gelatine had been liquefied, without any trace of growth of organisms. After 6 days the gelatine was liquefied to the depth of 11 mm. These experiments were repeated, with the addition of chloroform to the gelatine, and the results were the same—liquefaction of the

¹ “Les Trichophyties Humaines,” par Dr. Sabouraud. Paris, 1894; “Ueber die tieferen eiternden Schimmelerkrankungen der Haut,” von Dr. Rosenbach. Wiesbaden, 1894.

gelatine. These preliminary experiments gave evidence of the presence of an active enzyme in the ringworm cultures, which was capable of liquefying gelatine at a comparatively low temperature.

The experiments were continued in the following fashion. A tube of fresh gelatine was inoculated with the trichophyton. A second tube was inoculated with a few drops of gelatine, which had previously been liquefied by the ringworm organism. We had therefore a tube sown with ringworm alone, and one containing only the liquefied gelatine from a culture. The tubes were placed at blood heat, and examined on the following day. The tube inoculated directly with ringworm showed no growth, and the gelatine resolidified completely on being cooled down. The tube of gelatine to which the "enzyme gelatine" had been added was quite liquid, and remained so at room temperature. This experiment showed that the gelatine, which had been liquefied by the growth of the trichophyton, contained a soluble ferment, capable of liquefying gelatine independently of the cells that produced it. The amount present in a few drops was sufficient to completely liquefy about 10 c.c. of fresh gelatine. The gelatine, in which a growth of the trichophyton had barely started, did not yet contain this active ferment.

The experiments were continued at blood heat. A number of tubes of 10 per cent. gelatine were inoculated with ringworm. A further series were inoculated with a few drops of liquefied gelatine from a ringworm culture. The tubes were examined the next day. The tubes inoculated with the trichophyton showed neither a growth nor liquefaction of the gelatine. The tubes to which the "enzyme gelatine" had been added were completely liquefied. It was found that this complete liquefaction of the gelatine took place in 17-18 hours. The ferment acted more quickly and powerfully at blood heat than at 20° C. In the case of the tubes which had been kept at 20° C. the liquefaction of the gelatine was partial, whilst at blood heat the liquefaction was complete in the same space of time.

It will be seen that the peptonising enzyme secreted by this trichophyton is of a very active nature—a 10 per cent. solution of gelatine being liquefied within 18 hours. To render the experiments more conclusive, they were repeated with the addition of an antiseptic substance to the gelatine. A few drops of the oil of wintergreen were added to the tubes before inoculation. The oil retarded any growth of organisms, but did not interfere with the action of the ferment. The results were the same as detailed above, namely, complete liquefaction of the gelatine at blood heat by the enzyme. The liquefaction, however, is so rapid, that the addition of an antiseptic is hardly a necessary precaution.

The ferment action of the ringworm cultures was next tested at a temperature of 30° C. Five series of tubes were prepared in the following manner:—

1. Gelatine (10 per cent.) and ringworm organism and oil of wintergreen.

2. Gelatine and oil of wintergreen.
3. Gelatine and oil of wintergreen and liquefied gelatine from a ringworm culture.
4. Gelatine and ringworm organism.
5. Control tubes of uninoculated 10 per cent. gelatine.

The tubes were examined after having remained in an incubator at 30° C. for 18 hours. The only tubes that gave a positive result were those to which the "enzyme gelatine" had been added. The gelatine in these tubes was completely liquefied. The results corresponded with those obtained at blood heat, namely, complete liquefaction of the gelatine within 18 hours.

It was remarkable how small a quantity of the "enzyme gelatine" was necessary to produce liquefaction at 30° C. and at blood heat. Three droplets of "enzyme gelatine" caused a complete liquefaction of 10 per cent. gelatine at these temperatures.

The action of the "enzyme gelatine" was also tested on 15 per cent. gelatine. At blood heat, even this concentrated solution of gelatine was entirely liquefied.

If these results depend upon the presence of an enzyme in the gelatine, a temperature above 80° C. will interfere with or destroy its action. To test this, two tubes of gelatine, which had been completely liquefied by the trichophyton were used. One tube was exposed to a temperature of 100° C. for 2 minutes. A few drops of gelatine from the heated and unheated tubes were then added to respective tubes of fresh gelatine. The experiments with the heated gelatine gave entirely negative results. It produced no liquefaction of gelatine at 30° C. and at blood heat, though the tubes were left in the incubator for several days. On the other hand, the control tubes which had been inoculated with the unheated gelatine were liquefied.

The enzyme upon which the liquefying action on gelatine depends is thus rendered inactive when exposed to a temperature of 100° C. for 2 minutes.

A number of experiments were made with a simple 5 per cent. solution of gelatine in water, without any addition of peptone or beef-broth. The results were the same as in the previous experiments. The addition of a few drops of the "enzyme gelatine" produced a rapid, complete, and permanent liquefaction. This simple gelatine solution furnishes a very convenient means of rapidly testing the action of peptonising ferments generally.

Experiments were next made to determine whether the liquefying action of gelatine cultures of the trichophyton could be transmitted from tube to tube—whether, in other words, the enzyme would still act when greatly diluted. A few drops of the "enzyme gelatine" were added to sterile 10 per cent. gelatine, and the tubes placed at blood heat. When liquefaction was complete, a few drops were transferred to a second tube of gelatine. This second tube of gelatine was also liquefied at blood

heat. Some of the liquefied gelatine was then added to a third tube of gelatine, and so on. It was found that liquefaction still took place in the fourth tube of the series. This gave a fresh proof of the powerful nature of the peptonising enzyme secreted by the trichophyton, and of its capacity to act even when greatly diluted. The enzyme is also of a very stable nature. A few drops of gelatine from a ringworm culture 3 months old still exerted the liquefying action on gelatine.

These experiments seem to establish the following points. The *T. tonsurans* secretes a powerful peptonising enzyme, by means of which it is able to liquefy gelatine. The action of this enzyme can be demonstrated apart from the living cells that produce it. The enzyme rapidly liquefies 5, 10, and 15 per cent. solutions of gelatine. It can be greatly diluted without enfeebling its liquefying power. The enzyme is destroyed by boiling, but it is otherwise stable in its character, as it retains its activity for at least 3 months.

In the above experiments the action of the enzyme had been tested in neutral or alkaline media. Its action in acid media was next tested.

Nutrient gelatine was rendered acid by the addition of a 0·2 per cent. solution of hydrochloric acid. The trichophyton grew in this acid gelatine at 30° C., though its growth was slower than in neutral or alkaline gelatine. When liquefaction of the acid gelatine took place, a few drops were added to fresh gelatine. The tubes were kept at blood heat. After 2 days, the gelatine was partially liquefied. It was only on the third or fourth day that complete liquefaction took place.

The liquefied gelatine from an alkaline culture was added to the acidified gelatine. It was found that at blood heat complete liquefaction of this acid gelatine took place in 48 hours.

It will be seen that the peptonising enzyme is not only produced in an acid medium, but that it can also act in an acid medium. Its action, however, is slower and not so energetic as is the case when the medium has an alkaline reaction. It is undoubtedly sensitive to the presence of acids, and acts best when the medium is alkaline.

The *T. tonsurans* was next grown on fluid media, and the action of these cultures on gelatine was tested. Inoculations were made in a simple acid-broth infusion, and in a simple alkaline-broth infusion. In both cases a growth of the trichophyton was obtained. The broth was then added to a 5 per cent. solution of gelatine in water. The *alkaline* culture broth completely liquefied the 5 per cent. gelatine in 7 days. The *acid* culture broth produced no complete liquefaction of the 5 per cent. gelatine after the same lapse of time. The enzyme was therefore also produced in fluid broth cultures, and proved to be more active in the alkaline than in the acid broth. In this respect the results corresponded to those obtained with the acid and the alkaline gelatine. On the other hand, the enzyme produced in the alkaline gelatine was more energetic than that produced in the alkaline broth. The best

results were always obtained with alkaline gelatine cultures of the organisms.

Some experiments were made with a view of answering the following question:—Does the liquefied gelatine exercise any inimical action on other organisms in virtue of the enzyme which it contains? The experiments were carried out in the following manner. Gelatine which had been liquefied by the ringworm organism was boiled to destroy the ferment. A second series of tubes of the liquefied gelatine were not subjected to this heat. They therefore contained the active ferment. Both sets of tubes were then inoculated either with the *Staphylococcus pyogenes aureus* or the *Bacillus pyocyaneus*. Control cultures of these organisms were also made in ordinary nutrient gelatine. All the tubes were placed at blood heat. The results were negative as regards an inimical action of the enzyme on these organisms. The *Staphylococcus pyogenes aureus* and *B. pyocyaneus* grew well, not only in the control gelatine, but also in the heated and unheated tubes of “enzyme gelatine.” One could not therefore demonstrate any inimical action on these bacteria, through the presence of the active ferment in the cultures.

Experiments were made to determine whether the *T. tonsurans* produces a *diastatic ferment*. The organism was grown in watery solutions of starch of various concentrations. A growth was obtained in the starch water. It was then tested for the presence of sugar. No unmistakable evidence of a conversion of the starch into sugar could be obtained. The experiments led to the conclusion that the trichophyton does not produce a diastatic ferment.

On the other hand, the trichophyton grew well in a 5 per cent. solution of grape sugar, and also in a 5 per cent. solution of milk sugar. It also grew well in beef broth containing 2 per cent. of milk sugar or grape sugar. These sugars seemed rather to favour its growth than otherwise.

The trichophyton developed also in a 2 per cent. solution of cane sugar in 1 per cent. peptone water. The growth, however, was due mainly to the presence of the peptone, and not to the cane sugar. In simple watery solutions of cane sugar, with the addition of mineral salts, a slight growth was obtained, but it was feeble compared with the growth in the solutions of grape sugar and milk sugar.

The cane-sugar solutions, after inoculation with ringworm, reduced Fehling's solution slightly. This at first pointed to the presence of a weak inverting ferment in these cultures.

In an abstract of these results, published in the *British Medical Journal* (Sept. 22, 1894), it was stated that evidence was obtained of the production of a weak inverting ferment by the trichophyton. The experiments have since then been repeated several times. It was found that the cane sugar used was not chemically pure. It was therefore recrystallised, and the experiments were then repeated.

In these pure solutions of cane sugar, the trichophyton again gave a very feeble growth. The Fehling's solution was not reduced. Further, an examination of the solutions with the polarimeter gave no evidence of the presence of invert sugar. The trichophyton cannot, therefore, be said to produce an inverting ferment.

Finally, experiments were made as to the production of a *milk-curdling ferment*. The results were negative. The ringworm did not grow at all in milk, nor did cultures containing the active enzyme produce any curdling of pure milk.

The experiments have thus demonstrated solely the production of a very active peptonising ferment by the *T. tonsurans*. The question naturally occurred whether this enzyme does not aid the organisms when it attacks the tissue, by perhaps overcoming their resistance to the penetration of the hyphæ.

The action of the peptonising enzyme was first of all tested on fibrin. The "enzyme gelatine," to which a little chloroform had been added, was placed in test-tubes containing flakes of fibrin. The tubes were kept at 20°, 30°, and 37° C. The fluid in the tubes remained clear, and no solution or disintegration of the fibrin took place. The enzyme did not act at all upon the fibrin.

It might be that the enzyme would, however, act on keratin or bodies containing it. Hairs from the human scalp were subjected to the action of the peptonising enzyme. These experiments did not lead to any definite result. The action of the trichophyton and its ferment were finally tested on pure keratin. The keratin used was obtained from quills, and answered the following tests:—It was insoluble in water, dilute acids, dilute alkalis, alcohol, and ether. The first point was to ascertain whether the trichophyton would grow on a keratin soil. For this purpose a thick layer of keratin was placed on the surface of solid blood serum. The tubes were then sterilised. The keratin surface was then inoculated with the trichophyton, and the tubes incubated at 30° C. A growth was obtained under these conditions. The *T. tonsurans*, therefore, was capable of growing on a soil consisting entirely of keratin, and was apparently able to derive its nutriment from the keratin. One could not, however, observe that any solution of the keratin took place. The ringworm certainly grew on the keratin, but did not exhibit any dissolving action that might be attributed to the action of an enzyme.

Gelatine containing the active enzyme was added to the pure keratin, and here again no distinct evidence of solution of the keratin was obtained.

The growth of the trichophyton might, however, possibly affect the solubility of the keratin in some way. The keratin might, for example, be rendered more easily soluble than it is under ordinary conditions. Tubes of nutrient gelatine, containing keratin, were inoculated with the trichophyton, and incubated at 30° and 37° C. The organism grew equally well in the keratin-gelatine.

Control tubes of gelatine and keratin alone were also kept under the same conditions. After a good growth had appeared in the inoculated tubes the keratin was removed from the tubes, and its solubility, in a strong solution of caustic potash, was tested. The keratin from the ringworm tubes dissolved immediately on the alkaline solution being heated. The keratin from the control tubes required a prolonged boiling to dissolve it. The solubility of the keratin seemed, therefore, to have been affected by the growth of the trichophyton in the gelatine. This was as far as the experiments with keratin could be brought. The writer does not wish to lay too much stress on these experiments. They certainly suggest that the solubility of the keratin is affected, and the results seem worthy of being tested by other workers in the same field. At anyrate, it was interesting to note the fact that the trichophyton was capable of growing on a purely keratin soil.

It may be mentioned here that the trichophyton used in the above experiments was generally grown on the beer-wort agar, recommended by Dr Sabouraud. This medium furnishes an excellent soil for the *T. tonsurans*. It is prepared as follows:—Add 1–5 grms. of agar-agar to 100 grms. of beer-wort; then boil and filter hot. Place the filtrate in test-tubes or Erlenmeyer flasks, and sterilise in the usual way. The beer-wort agar solidifies on cooling.

